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Preparation of odorant and buffer solutions

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An abbreviated version of this protocol was published in Science Advances in Mar 2023

Functional imaging and quantification of multineuronal olfactory responses in C. elegans

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Detailed protocol

Protocols for preparing CTX buffer (5 mM KH_2PO_4/K_2HPO_4 pH 6.0, 1mM $CaCl_2$, 1mM $MgSO_4$, and 50mM NaCl. Odors were diluted in CTX and the osmolarity of these solutions was adjusted with sorbitol to 350 mOsm/L to match the internal osmotic pressure of *C. elegans* and prevent osmotic shock.

CTX buffer protocol:

Materials
DI water
Pipette
Laboratory scale
Stir bar
pH meter
1 M KPO_4 buffer (KH_2PO_4/K_2HPO_4 pH 6.0)
1 M $CaCl_2$
1 M $MgSO_4$
NaCl
Sorbitol
Osmometer
**All salts were sourced from Sigma Aldrich. Sorbitol was sourced from Alfa Aesar.*

1. If not already on hand, first separately prepare the following solutions: 1 M $CaCl_2$, 1 M $MgSO_4$, and 1 M KPO_4 buffer (KH_2PO_4/K_2HPO_4, with a measured pH of 6.0).
2. To make 1 L of CTX buffer with no sorbitol, first add 500 mL DI water to a suitable container. Use a pipette to add 5 mL 1 M KPO_4 buffer, 1 mL 1 M $CaCl_2$, 1 mL 1 M $MgSO_4$, and 0.05 mole NaCl (2.92 g). Then add DI water until the solution reaches 1 L total volume.
3. Stir to dissolve the salt. CTX is stable prior to the addition of sorbitol.
4. To prepare a small quantity CTX buffer with sorbitol in preparation for odorant dilution, first compute the required amount of sorbitol to achieve the desired osmolarity.
    a. Most odorants are nonionic compounds and thus only contribute 1 osmole of solute per 1 mole of solute. For salts, this ratio changes.
    b. Use an osmometer to measure the osmolarity of the CTX buffer without sorbitol (~125 mOsm/L).
    c. Add to the buffer osmolarity add the osmolarity contributed by the concentration of the odorant (negligible at low dilutions), then determine the amount of sorbitol (182.17 g/mol) necessary to bring the total osmolarity to 350 mOsm/L when the contributions of the CTX buffer, odorant, and sorbitol are combined. In the case that the odorant concentration is negligible, sorbitol should be added at ~225 mM (40.9 g/L).
5. To prepare 200 mL of CTX buffer with sorbitol, combine 200 mL of prepared CTX buffer with the requisite amount of sorbitol. Stir until the sorbitol has completely dissolved.
6. Confirm with an osmometer that the final osmolarity of the solution is correct.

Odor dilution protocol:

Materials
DI water
Pipette
Micropipettes
Pure soluble odorants (liquid form)
CTX buffer with sorbitol
Glass bottles for odorant storage
**All pure odorants were sourced from Sigma Aldrich. Refer to the supplement of Lin et al., 2023 for a full listing of the odors used.*

1. Use a pipette to add 10 mL CTX buffer with sorbitol in a clean glass bottle. Use a micropipette to add 1 uL pure odorant to the bottle (10^{-4} dilution). Stir thoroughly to combine. Let any bubbles that may have formed during the stirring process settle before proceeding.
2. Perform serial dilution: In a second glass bottle, add 9 mL CTX buffer with sorbitol. Add from the first bottle 1 mL of 10^{-4} dilution and stir well. This makes the 10^{-5} dilution.
3. Repeat the serial dilution until you reach the desired minimum concentration. Note that if you are spanning a large range of concentrations, the amount of sorbitol may change. Thus, you may have to prepare different CTX + sorbitol buffers for different concentrations.
4. To minimize odorant cross-contamination, glass bottles were not reused for different odors or different concentrations. Odor solutions not consumed in experiments were replaced after 2 weeks to mitigate changes in odor solution concentration due to evaporation of volatile compounds. Particularly volatile odorants were replaced even more frequently.

Related files

Preparation of odorant and buffer solutions.pdf

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Lin, A. , Pehlevan, C. , Zhen, M. and Samuel, A. (2023). Preparation of odorant and buffer solutions. Bio-protocol Preprint. bio-protocol.org/prep2190.

2. Lin, A., Qin, S., Casademunt, H., Wu, M., Hung, W., Cain, G., Tan, N. Z., Valenzuela, R., Lesanpezeshki, L., Venkatachalam, V., Pehlevan, C., Zhen, M. and Samuel, A. D.(2023). Functional imaging and quantification of multineuronal olfactory responses in C. elegans. Science Advances 9(9). DOI: 10.1126/sciadv.ade1249